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Sequence Listing could not be accepted due to errors.

See attached Validation Report.

If you need help call the Patent Electronic Business Center at (866)  
217-9197 (toll free).

Reviewer: Anne Corrigan

Timestamp: [year=2008; month=1; day=23; hr=13; min=45; sec=43; ms=375; ]

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\*\*\*\*\*

Reviewer Comments:

<120> A METHOD FOR CREATING A FUNCTIONALLY ACTIVE CHIMERIC TYPE IIG  
RESTRICTION ENDONUCLEASE (as amended)

The first line of the above <120> response exceeds the Sequence Rules'  
required 72-character line (this includes white spaces). Please insert  
a hard return after "TYPE" on the first line, and align the second line.

(from Sequence 2)

Val	Asp	Glu	Ala	Leu	Leu	Ile	Lys	Tyr	His	Gly	Phe	Ser	Glu	Lys	Glu
	515					520						525			

Val	Lys	Gln	Leu	Arg	Gly	Ile	Trp	Lys	Lys	Leu	Ser	Gln	Arg	Arg	Asn
	530				535					540					

Asn	Arg	Thr	Lys	Lys
545				

Please delete the excess blank lines above: only one blank line should  
separate each amino acid line.

<210> 38  
<211> 103  
<212> PRT  
<213> artificial

<220>

<223> segment of protein sequence of catechol O-methyltransferase

<400> 39

Please change the above <210> response to "39." <210> 38 was already shown.

\*\*\*\*\*

Application No: 10800946 Version No: 2.0

**Input Set:****Output Set:**

**Started:** 2008-01-09 14:09:09.990  
**Finished:** 2008-01-09 14:09:12.054  
**Elapsed:** 0 hr(s) 0 min(s) 2 sec(s) 64 ms  
**Total Warnings:** 25  
**Total Errors:** 2  
**No. of SeqIDs Defined:** 43  
**Actual SeqID Count:** 43

Error code	Error Description
W 213	Artificial or Unknown found in <213> in SEQ ID (19)
W 213	Artificial or Unknown found in <213> in SEQ ID (20)
W 213	Artificial or Unknown found in <213> in SEQ ID (21)
W 213	Artificial or Unknown found in <213> in SEQ ID (22)
W 213	Artificial or Unknown found in <213> in SEQ ID (23)
W 213	Artificial or Unknown found in <213> in SEQ ID (24)
W 213	Artificial or Unknown found in <213> in SEQ ID (25)
W 213	Artificial or Unknown found in <213> in SEQ ID (26)
W 213	Artificial or Unknown found in <213> in SEQ ID (27)
W 213	Artificial or Unknown found in <213> in SEQ ID (28)
W 213	Artificial or Unknown found in <213> in SEQ ID (29)
W 213	Artificial or Unknown found in <213> in SEQ ID (30)
W 213	Artificial or Unknown found in <213> in SEQ ID (31)
W 213	Artificial or Unknown found in <213> in SEQ ID (32)
W 213	Artificial or Unknown found in <213> in SEQ ID (33)
W 213	Artificial or Unknown found in <213> in SEQ ID (34)
W 213	Artificial or Unknown found in <213> in SEQ ID (35)
W 213	Artificial or Unknown found in <213> in SEQ ID (36)
W 213	Artificial or Unknown found in <213> in SEQ ID (37)
W 213	Artificial or Unknown found in <213> in SEQ ID (38)

**Input Set:**

**Output Set:**

**Started:** 2008-01-09 14:09:09.990  
**Finished:** 2008-01-09 14:09:12.054  
**Elapsed:** 0 hr(s) 0 min(s) 2 sec(s) 64 ms  
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Error code	Error Description
	This error has occurred more than 20 times, will not be displayed
E 212	Invalid Sequence ID Number; Expected 39 as next SeqID but skipped
E 212	Invalid Sequence ID Number; Expected 39 as next SeqID but skipped

# SEQUENCE LISTING

<110> Xu, Shuang-yong  
Kobbe, Daniela  
Zhu, Zhenyu  
Samuelson, James

<120> A METHOD FOR CREATING A FUNCTIONALLY ACTIVE CHIMERIC TYPE IIG RESTRICTION ENDONUCLEASE  
(as amended)

<130> NEB-183-CIP

<140> 10800946

<141> 2004-03-15

<150> 10/150,028

<151> 2002-05-17

<150> 09/693,146

<151> 2000-07-02

<160> 43

<170> PatentIn version 3.2 (1-26) and 3.4 (27-43)

<210> 1

<211> 1650

<212> DNA

<213> Bacillus pumilus

<220>

<221> CDS

<222> (1)..(1650)

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1 5 10 15

tat tac acc cct aaa gtt att gct gac ttt tta tgt caa tgg agt att 96  
Tyr Tyr Thr Pro Lys Val Ile Ala Asp Phe Leu Cys Gln Trp Ser Ile  
20 25 30

caa gat gac aca aag agt gta ctt gaa ccc agt tgt gga gat ggt aat 144  
Gln Asp Asp Thr Lys Ser Val Leu Glu Pro Ser Cys Gly Asp Gly Asn  
35 40 45

ttt att gaa tgc gca ata ctt agg ttc aaa gaa ctt agt ata gat aat 192  
Phe Ile Glu Ser Ala Ile Leu Arg Phe Lys Glu Leu Ser Ile Asp Asn  
50 55 60

gaa caa ctt aaa gga aga att aca gga gta gag cta att gaa gaa gaa 240  
Glu Gln Leu Lys Gly Arg Ile Thr Gly Val Glu Leu Ile Glu Glu Glu  
65 70 75 80

gct ttg aaa gtt caa aat cga gca aat gag ttg ggg gtt gat aaa aac 288  
Ala Leu Lys Val Gln Asn Arg Ala Asn Glu Leu Gly Val Asp Lys Asn

tca ata gta aat agt gac ttc ttt caa ttt gta aaa gat aat aag aat	336
Ser Ile Val Asn Ser Asp Phe Phe Gln Phe Val Lys Asp Asn Lys Asn	
100 105 110	
aaa aaa ttt gat act att att ggt aat cca cca ttc ata aga tac caa	384
Lys Lys Phe Asp Thr Ile Ile Gly Asn Pro Pro Phe Ile Arg Tyr Gln	
115 120 125	
aac ttt cct gaa gag cat cgt agt ata gcc atg gaa atg atg gag gaa	432
Asn Phe Pro Glu Glu His Arg Ser Ile Ala Met Glu Met Met Glu Glu	
130 135 140	
cta ggt tta aaa cct aat aaa ctt aca aat atc tgg gtt cca ttt cta	480
Leu Gly Leu Lys Pro Asn Lys Leu Thr Asn Ile Trp Val Pro Phe Leu	
145 150 155 160	
gtg gta tct gct aca tta ctt aat gaa caa gga aag atg gct atg gtt	528
Val Val Ser Ala Thr Leu Leu Asn Glu Gln Gly Lys Met Ala Met Val	
165 170 175	
ata ccg gct gaa tta ttt cag gta aag tat gca gca gaa aca aga att	576
Ile Pro Ala Glu Leu Phe Gln Val Lys Tyr Ala Ala Glu Thr Arg Ile	
180 185 190	
ttt tta tca aag ttt ttc gat cgt atc act ata att aca ttt gaa aaa	624
Phe Leu Ser Lys Phe Phe Asp Arg Ile Thr Ile Ile Thr Phe Glu Lys	
195 200 205	
ctt gtt ttt gaa aat atc caa cag gaa gtt ata cta ctt ctt tgt gaa	672
Leu Val Phe Glu Asn Ile Gln Gln Glu Val Ile Leu Leu Leu Cys Glu	
210 215 220	
aag aaa gtt aat aaa ggt aaa gga att cgg gtt att gaa tgc gag aac	720
Lys Lys Val Asn Lys Gly Lys Gly Ile Arg Val Ile Glu Cys Glu Asn	
225 230 235 240	
tta gat gga tta aat tcc att gat ttt gta gct ata aat ggt tca aat	768
Leu Asp Gly Leu Asn Ser Ile Asp Phe Val Ala Ile Asn Gly Ser Asn	
245 250 255	
gtt aaa cct att gaa cac cgt act gaa aag tgg aca aag tat ttc tta	816
Val Lys Pro Ile Glu His Arg Thr Glu Lys Trp Thr Lys Tyr Phe Leu	
260 265 270	
aac gaa gat gaa ata ctt ctt tta cag agt tta aag gaa gac aaa cgc	864
Asn Glu Asp Glu Ile Leu Leu Leu Gln Ser Leu Lys Glu Asp Lys Arg	
275 280 285	
gtt aaa aat tgt aat gac tat ttt aag aca gaa gtt ggc tta gtt act	912
Val Lys Asn Cys Asn Asp Tyr Phe Lys Thr Glu Val Gly Leu Val Thr	
290 295 300	
gga cga aac gaa ttc ttt atg atg aaa gaa aac caa gta aaa gaa tgg	960
Gly Arg Asn Glu Phe Phe Met Met Lys Glu Asn Gln Val Lys Glu Trp	

305	310	315	320	
aat cta gaa gaa tat aca ata cct gtt aca ggt agg tcc aat cag tta				1008
Asn Leu Glu Glu Tyr Thr Ile Pro Val Thr Gly Arg Ser Asn Gln Leu				
325		330	335	
aaa ggt ata aca ttt aca gaa aat gat ttt cat gaa aat tca atg gaa				1056
Lys Gly Ile Thr Phe Thr Glu Asn Asp Phe His Glu Asn Ser Met Glu				
340		345	350	
caa aag gca att cac cta ttt ttg cca cca gat gaa gat ttt gaa aag				1104
Gln Lys Ala Ile His Leu Phe Leu Pro Pro Asp Glu Asp Phe Glu Lys				
355		360	365	
tta ccg att gag tgt caa aat tat atc aag tat ggg gaa gaa aaa ggc				1152
Leu Pro Ile Glu Cys Gln Asn Tyr Ile Lys Tyr Gly Glu Glu Lys Gly				
370		375	380	
ttc cat caa ggc tat aaa acc aga att aga aaa cgt tgg tat ata act				1200
Phe His Gln Gly Tyr Lys Thr Arg Ile Arg Lys Arg Trp Tyr Ile Thr				
385		390	395	400
cca tct aga tgg gtt cca gat gct ttt gct tta aga cag gtt gat ggc				1248
Pro Ser Arg Trp Val Pro Asp Ala Phe Ala Leu Arg Gln Val Asp Gly				
405		410	415	
tat cca aaa cta att tta aat gaa acc gac gct tct tct act gat aca				1296
Tyr Pro Lys Leu Ile Leu Asn Glu Thr Asp Ala Ser Ser Thr Asp Thr				
420		425	430	
att cat agg gtt aga ttt aaa gaa ggt ata aat gaa aag tta gcc gta				1344
Ile His Arg Val Arg Phe Lys Glu Gly Ile Asn Glu Lys Leu Ala Val				
435		440	445	
gtt tca ttt ttg aac tca ctc act ttt gca tct tca gaa ata acg ggg				1392
Val Ser Phe Leu Asn Ser Leu Thr Phe Ala Ser Ser Glu Ile Thr Gly				
450		455	460	
aga agt tat ggt ggt ggt gtt atg aca ttc gaa cca act gaa att gga				1440
Arg Ser Tyr Gly Gly Gly Val Met Thr Phe Glu Pro Thr Glu Ile Gly				
465		470	475	480
gaa atc cta ata cct tcc ttt gat aac tta tcc att gat ttt gat aaa				1488
Glu Ile Leu Ile Pro Ser Phe Asp Asn Leu Ser Ile Asp Phe Asp Lys				
485		490	495	
att gat gcc tta att cga gaa aag gag att gaa aaa gtc ctt gat att				1536
Ile Asp Ala Leu Ile Arg Glu Lys Glu Ile Glu Lys Val Leu Asp Ile				
500		505	510	
gtt gat gaa gct tta ctt ata aaa tat cat ggg ttt agt gag aaa gaa				1584
Val Asp Glu Ala Leu Leu Ile Lys Tyr His Gly Phe Ser Glu Lys Glu				
515		520	525	
gta aaa cag ctt cga ggg ata tgg aag aaa ctt tct cag aga aga aac				1632
Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn				

530

535

540

aat aga acg aag aaa taa

1650

Asn Arg Thr Lys Lys

545

550

&lt;210&gt; 2

&lt;211&gt; 549

&lt;212&gt; PRT

&lt;213&gt; Bacillus pumilus

&lt;400&gt; 2

Met Asn Gln Leu Ile Glu Asn Val Asn Leu Gln Lys Leu Arg Gly Gly

1

5

10

15

Tyr Tyr Thr Pro Lys Val Ile Ala Asp Phe Leu Cys Gln Trp Ser Ile

20

25

30

Gln Asp Asp Thr Lys Ser Val Leu Glu Pro Ser Cys Gly Asp Gly Asn

35

40

45

Phe Ile Glu Ser Ala Ile Leu Arg Phe Lys Glu Leu Ser Ile Asp Asn

50

55

60

Glu Gln Leu Lys Gly Arg Ile Thr Gly Val Glu Leu Ile Glu Glu Glu

65

70

75

80

Ala Leu Lys Val Gln Asn Arg Ala Asn Glu Leu Gly Val Asp Lys Asn

85

90

95

Ser Ile Val Asn Ser Asp Phe Phe Gln Phe Val Lys Asp Asn Lys Asn

100

105

110

Lys Lys Phe Asp Thr Ile Ile Gly Asn Pro Pro Phe Ile Arg Tyr Gln

115

120

125

Asn Phe Pro Glu Glu His Arg Ser Ile Ala Met Glu Met Met Glu Glu

130

135

140

Leu Gly Leu Lys Pro Asn Lys Leu Thr Asn Ile Trp Val Pro Phe Leu

145

150

155

160

Val Val Ser Ala Thr Leu Leu Asn Glu Gln Gly Lys Met Ala Met Val

165

170

175

Ile Pro Ala Glu Leu Phe Gln Val Lys Tyr Ala Ala Glu Thr Arg Ile

180

185

190

Phe Leu Ser Lys Phe Phe Asp Arg Ile Thr Ile Ile Thr Phe Glu Lys

195

200

205

Leu Val Phe Glu Asn Ile Gln Gln Glu Val Ile Leu Leu Leu Cys Glu

210

215

220

Lys Lys Val Asn Lys Gly Lys Gly Ile Arg Val Ile Glu Cys Glu Asn



225		230		235		240
Leu Asp Gly Leu Asn Ser Ile Asp Phe Val Ala Ile Asn Gly Ser Asn						
	245		250		255	
Val Lys Pro Ile Glu His Arg Thr Glu Lys Trp Thr Lys Tyr Phe Leu						
	260		265		270	
Asn Glu Asp Glu Ile Leu Leu Leu Gln Ser Leu Lys Glu Asp Lys Arg						
	275		280		285	
Val Lys Asn Cys Asn Asp Tyr Phe Lys Thr Glu Val Gly Leu Val Thr						
	290		295		300	
Gly Arg Asn Glu Phe Phe Met Met Lys Glu Asn Gln Val Lys Glu Trp						
305		310		315		320
Asn Leu Glu Glu Tyr Thr Ile Pro Val Thr Gly Arg Ser Asn Gln Leu						
	325		330		335	
Lys Gly Ile Thr Phe Thr Glu Asn Asp Phe His Glu Asn Ser Met Glu						
	340		345		350	
Gln Lys Ala Ile His Leu Phe Leu Pro Pro Asp Glu Asp Phe Glu Lys						
	355		360		365	
Leu Pro Ile Glu Cys Gln Asn Tyr Ile Lys Tyr Gly Glu Glu Lys Gly						
	370		375		380	
Phe His Gln Gly Tyr Lys Thr Arg Ile Arg Lys Arg Trp Tyr Ile Thr						
385		390		395		400
Pro Ser Arg Trp Val Pro Asp Ala Phe Ala Leu Arg Gln Val Asp Gly						
	405		410		415	
Tyr Pro Lys Leu Ile Leu Asn Glu Thr Asp Ala Ser Ser Thr Asp Thr						
	420		425		430	
Ile His Arg Val Arg Phe Lys Glu Gly Ile Asn Glu Lys Leu Ala Val						
	435		440		445	
Val Ser Phe Leu Asn Ser Leu Thr Phe Ala Ser Ser Glu Ile Thr Gly						
	450		455		460	
Arg Ser Tyr Gly Gly Gly Val Met Thr Phe Glu Pro Thr Glu Ile Gly						
465		470		475		480
Glu Ile Leu Ile Pro Ser Phe Asp Asn Leu Ser Ile Asp Phe Asp Lys						
	485		490		495	
Ile Asp Ala Leu Ile Arg Glu Lys Glu Ile Glu Lys Val Leu Asp Ile						
	500		505		510	
Val Asp Glu Ala Leu Leu Ile Lys Tyr His Gly Phe Ser Glu Lys Glu						
	515		520		525	

Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn  
530 535 540

Asn Arg Thr Lys Lys  
545

<210> 3  
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<212> DNA  
<213> Bacillus pumilus

<220>  
<221> CDS  
<222> (1)..(3030)

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ttt tta aaa cca act tat aat gaa act caa cta agg aat gat ttt ata 96  
Phe Leu Lys Pro Thr Tyr Asn Glu Thr Gln Leu Arg Asn Asp Phe Ile  
20 25 30  
  
gac cca ctt cta aaa tct tta gga tgg gat gtt gat aat acc aaa gga 144  
Asp Pro Leu Leu Lys Ser Leu Gly Trp Asp Val Asp Asn Thr Lys Gly  
35 40 45  
  
aaa aca cat att cta aga gat gtc att caa gaa gaa tac ata gaa ata 192  
Lys Thr His Ile Leu Arg Asp Val Ile Gln Glu Glu Tyr Ile Glu Ile  
50 55 60  
  
aaa gat gag gag aca aag aaa aat cca gat tat aca ctt cgt ata aac 240  
Lys Asp Glu Glu Thr Lys Lys Asn Pro Asp Tyr Thr Leu Arg Ile Asn  
65 70 75 80  
  
ggg acg aga aag ctg ttt gta gag gtt aag aaa ccg tct ttt aat att 288  
Gly Thr Arg Lys Leu Phe Val Glu Val Lys Lys Pro Ser Phe Asn Ile  
85 90 95  
  
ttg aaa tca gct aaa gca gcc ttc caa aca aga aga tat ggt tgg agt 336  
Leu Lys Ser Ala Lys Ala Ala Phe Gln Thr Arg Arg Tyr Gly Trp Ser  
100 105 110  
  
gct aac ctt ggt att tca gta ctt aca aat ttc gag cat cta gtt att 384  
Ala Asn Leu Gly Ile Ser Val Leu Thr Asn Phe Glu His Leu Val Ile  
115 120 125  
  
tat gat tgt aga tat acg cct gac aaa tcc gac aat gaa cat att gct 432  
Tyr Asp Cys Arg Tyr Thr Pro Asp Lys Ser Asp Asn Glu His Ile Ala  
130 135 140  
  
aga tat aaa gtt ttc tct tac gag gaa tat gaa gaa gca ttt gat gaa 480

Arg Tyr Lys Val Phe Ser Tyr Glu Glu Tyr Glu Glu Ala Phe Asp Glu	
145	150 155 160
ata aag gat ata att tca tat gag tca gcc aac tca ggt gct ctg gac	528
Ile Lys Asp Ile Ile Ser Tyr Glu Ser Ala Asn Ser Gly Ala Leu Asp	
165 170 175	
gaa atg ttt gat gta aat aca aga gtt ggt gaa acg ttt gac gag tat	576
Glu Met Phe Asp Val Asn Thr Arg Val Gly Glu Thr Phe Asp Glu Tyr	
180 185 190	
ttt tta cag caa att gag aat tgg cgc gaa aag cta gct aaa act gca	624
Phe Leu Gln Gln Ile Glu Asn Trp Arg Glu Lys Leu Ala Lys Thr Ala	
195 200 205	
att aaa aat aac acc gaa tta ggt gaa gag gac gtc aat ttt att gtc	672
Ile Lys Asn Asn Thr Glu Leu Gly Glu Glu Asp Val Asn Phe Ile Val	
210 215 220	
caa aga cta tta aac aga att att ttt ctt aga gtt tgt gaa gat aga	720
Gln Arg Leu Leu Asn Arg Ile Ile Phe Leu Arg Val Cys Glu Asp Arg	
225 230 235 240	
acc att gaa aaa tat gaa aca att aaa agt ata aaa aac tat gag gaa	768
Thr Ile Glu Lys Tyr Glu Thr Ile Lys Ser Ile Lys Asn Tyr Glu Glu	
245 250 255	
tta aaa gat ctg ttt caa aag tct gat agg aaa ttt aat tca ggt ctc	816
Leu Lys Asp Leu Phe Gln Lys Ser Asp Arg Lys Phe Asn Ser Gly Leu	
260 265 270	
ttt gac ttc ata gat gat acg ctc ttg ctt gag gtt gaa att gat tcg	864
Phe Asp Phe Ile Asp Asp Thr Leu Leu Leu Glu Val Glu Ile Asp Ser	
275 280 285	
aat gta ttg ata gaa att ttt agt gat tta tat ttc cca caa agc cca	912
Asn Val Leu Ile Glu Ile Phe Ser Asp Leu Tyr Phe Pro Gln Ser Pro	
290 295 300	
tat gat ttt tct gtt gtc gat cca aca ata tta agc cag ata tat gaa	960
Tyr Asp Phe Ser Val Val Asp Pro Thr Ile Leu Ser Gln Ile Tyr Glu	
305 310 315 320	
cgt ttt cta ggt caa gaa ata att ata gag tca ggt ggt aca ttt cac	1008
Arg Phe Leu Gly Gln Glu Ile Ile Ile Glu Ser Gly Gly Thr Phe His	
325 330 335	
att acg gag tca cca gaa gtt gcg gcg tcc aat ggt gtt gtt cca act	1056
Ile Thr Glu Ser Pro Glu Val Ala Ala Ser Asn Gly Val Val Pro Thr	
340 345 350	
cca aaa att atc gtc gaa cag ata gtg aaa gac act tta acg ccc ctt	1104
Pro Lys Ile Ile Val Glu Gln Ile Val Lys Asp Thr Leu Thr Pro Leu	
355 360 365	
acg gaa ggc aaa aaa ttt aat gag cta tgt aac tta aaa ata gca gat	1152
Thr Glu Gly Lys Lys Phe Asn Glu Leu Cys Asn Leu Lys Ile Ala Asp	

370

375

380

ata tgt tgt gga tca gga act ttc cta att tca agt tat gac ttt cta 1200  
Ile Cys Cys Gly Ser Gly Thr Phe Leu Ile Ser Ser Tyr Asp Phe Leu  
385 390 395 400

gta gag aaa gta atg gaa aag ata ata gaa gag aac atc gat gat tca 12